PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/014306	06.05.2004	06.05.2003

International Patent Classification (IPC) or both national classification and IPC
C07K14/34, C12N15/62, A61P35/00, A61P35/02, A61K38/19, C07K14/535

Applicant
THE GOVERNMENT OF THE UNITED STATES, AS ...

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/014306

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 24-48

because:

☒ the said international application, or the said claims Nos. 24-48 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-49
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-49
Industrial applicability (IA)	Yes:	Claims	1-23, 49
	No:	Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

D1: LIU S ET AL: "Targeting of tumor cells by cell surface urokinase plasminogen activator-dependent anthrax toxin" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 276, no. 21, 25 May 2001 (2001-05-25), pages 17976-17984, XP002974279 ISSN: 0021-9258

D2: LIU SHIHUI ET AL: "Tumor cell-selective cytotoxicity of matrix metalloproteinase-activa ted anthrax toxin" CANCER RESEARCH, vol. 60, no. 21, 1 November 2000 (2000-11-01), pages 6061-6067, XP002311242 ISSN: 0008-5472

D3: FRANKEL ARTHUR E ET AL: "Phase I trial of a novel diphtheria toxin/granulocyte macrophage colony-stimulating factor fusion protein (DT388GMCSF) for refractory or relapsed acute myeloid leukemia." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. MAY 2002, vol. 8, no. 5, May 2002 (2002-05), pages 1004-1013, XP002311243 ISSN: 1078-0432

Introduction

The present application discloses fusion proteins comprising diphtheria toxin whose native furin recognition site has been replaced by matrix metalloprotein (MMP) or plasminogen activator recognition cleavage site and a heterologous polypeptide that binds a protein expressed on the cell to be targeted (cancer cell).

Section III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 24-48 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Section V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Claim 1 is directed to a nucleic acid encoding a diphtheria toxin fusion protein comprising residues 1-388 of diphtheria toxin (DT), wherein the native furin cleavage site has been substituted for a cleavage site for a matrix

metalloproteinase (MMP) or a plasminogen activator (PA) and a heterologous polypeptide that specifically binds to a protein overexpressed on the surface of a cell.

Document D3 can be considered the closest prior art. It discloses the diphtheria toxin fusion protein DT388GMCSF, wherein residues 1-388 of DT are fused to GM-CSF in order to target acute myeloid leukemic cells which overexpress the receptor for GM-CSF. The difference to the present application is that substitution of the native furin cleavage site in DT is not foreseen. The technical problem can thus be formulated as the provision of more specific DT fusion proteins.

Document D1 discloses another cancer cell targeting strategy which makes use of a mutated anthrax toxin-protein Ag (PrAg) wherein the furin cleavage site is replaced by sequences cleaved specifically by uPA. This document furthermore reviews other targeting strategies such as that of D3 and suggests to overcome the high toxicity observed in such strategies by increasing specificity through use of the two targeting strategies together (page 17984 left column lines 7-13): i.e. targeting to tumour cell protein by fusion to peptide that binds to protein overexpressed at the surface of the targeted cells; and making the toxin activation dependent on cell surface PA system (also specifically expressed at the surface of tumour cells). Document D2 has a similar teaching as D1 but uses a MMP cleavage site instead of an uPA one; this document also suggests combining the two targeting strategies (page 6066 right column last 6 lines).

It thus appears that the person skilled in the art would just have to combine the teachings of D3 with those of D1 or D2 in order to arrive at the invention.

Claim 1 is thus considered to lack an inventive step (Art. 33(3) PCT).

For the same reasons further claims 1-10, 12-19, 21 and 22, which further specificy on the components and cleavage sequences of the fusion protein of claim 1, also do not involve an inventive step (Art. 33(3) PCT).

2. Claims 11, 20, and 23-49, which are directed to vectors, host cells, pharmaceutical compositions and methods of treatment, are considered to be mere technical variations of a non-inventive subject-matter. These claims thus also lack inventive step (Art. 33(3) PCT).